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Enhancers are genes that express organizational RNAs

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A longstanding enigma in molecular biology is the lack of scaling of protein-coding genes with developmental complexity, referred to as the g-value paradox. On the other hand, a feature of the evolution of multicellular organisms is the emergence of genetic loci termed "enhancers," which control the spatiotemporal patterns of gene expression during development. Enhancer action has been widely interpreted in terms of an early model that postulated that transcription factors bound at enhancers are brought into juxtaposition with the promoters of target genes. This model tacitly assumed that there is no trans-acting gene product of enhancers, but subsequent studies have shown that enhancers are transcribed in the cells in which they are active. Like protein-coding genes, enhancers produce short bidirectional transcripts and long alternatively spliced RNAs, albeit at lower levels due to their transitory and cell-specific regulatory functions. The evidence indicates that long noncoding RNAs (lncRNAs) expressed from enhancers (elncRNAs) guide the formation of phase-separated transcriptional hubs and the epigenetic modifications to direct cell fate decisions during animal and plant ontogeny. Many, and likely most, lncRNAs are elncRNAs, which should be recognized as a *bona fide* class of gene products alongside mRNAs, rRNAs, tRNAs, snoRNAs, miRNAs and others of established function, with sequences specifying elncRNAs comprising an increasing fraction of genomic information as developmental complexity increases.

KEYWORDS

long noncoding RNA, elncRNA, development, chromatin looping, chromatin modification

Introduction

Enhancers are genomic sequences in animals and plants that control developmental cell-type-specific spatiotemporal expression patterns of a subset of genes in their neighborhood (Hnisz et al., 2013; Shlyueva et al., 2014; Arnold et al., 2020). Enhancers can be located hundreds of kilobases away from their target genes and are (local) position and orientation-independent (Schoenfelder and Fraser, 2019; Arnold et al., 2020).

Enhancer activity was first observed by Ed Lewis and others in their early studies of the *bithorax* complex of *Drosophila melanogaster* (Maeda and Karch, 2006), although it was only in the early 1980s that the term was coined to describe the unexpected ability of certain SV40 virus sequences to increase the expression of a β-globin gene (Banerji et al., 1981). Many tissue-specific enhancers, also referred to as "locus control regions" (Li et al., 2002),

Abbreviations: lncRNA, long noncoding RNA; PSD, phase-separated domain; TAD, topologically-associated domain; IDR, intrinsically disordered region; eRNA, enhancer-derived RNA; elncRNA, enhancer-derived lncRNA.

were subsequently identified in mammalian immunoglobulin and globin gene loci, as well as in other genes that show restricted expression patterns during development, initially using cloning and deletion approaches (Banerji et al., 1983; Gillies et al., 1983; Whiting et al., 1991; Miyagi et al., 2004; Park et al., 2004; Perry et al., 2011) or insertions of transposons with reporter genes ("enhancer trapping") in *Drosophila* (O'Kane and Gehring, 1987; Galloni et al., 1993; McCall et al., 1994; Stathopoulos et al., 2002), plants (Springer, 2000) and other vertebrates (Trinh and Fraser, 2013), lately extended to high throughput CRISPR mutagenesis (Canver et al., 2015; Fulco et al., 2019; Gasperini et al., 2019).

Attempts have been made to typify known enhancers and identify others by their molecular features, including the location of presumed signature proteins (the "transcriptional co-activators" P300/CBP and Mediator), characteristic histone modifications, nucleosome-depleted regions, chromatin topology and/or the expression of "enhancer RNAs" (eRNAs) (Heintzman et al., 2007; Heintzman et al., 2009; De Santa et al., 2010; Wang D et al., 2011; Shen et al., 2012; Whyte et al., 2013; Wu et al., 2014; Kim et al., 2015; Pradeepa et al., 2016; Fulco et al., 2019; Osmala and Lähdesmäki, 2020). Although these features yield somewhat different prediction sets (Hnisz et al., 2013; Heidari et al., 2014; Shlyueva et al., 2014; Rickels and Shilatifard, 2018; Fulco et al., 2019; Halfon, 2019), they have been used to estimate that there are hundreds of thousands of enhancers in the human genome (De Santa et al., 2010; Dunham et al., 2012; Shen et al., 2012; Thurman et al., 2012; Zhu et al., 2013; Andersson et al., 2014; Heidari et al., 2014; Arnold et al., 2020; Chen and Liang, 2020), clusters of which have been dubbed "super-enhancers," "stretch enhancers" or "enhancer jungles" (Hnisz et al., 2013; Parker et al., 2013; Whyte et al., 2013; Pott and Lieb, 2015; Wang et al., 2019; Chen and Liang, 2020; Li and Ovcharenko, 2020).

The appearance of enhancers is associated with the emergence of animal and plant multicellularity and phenotypic diversity (Rubinstein and de Souza, 2013; Villar et al., 2015; Sebé-Pedrós et al., 2016; Rebeiz and Tsiantis, 2017), neuronal expansion in vertebrates (Closser et al., 2021) and the recent evolution of primates (Lagha et al., 2012; Glinsky and Barakat, 2019; Tejada-Martinez et al., 2021). Positive selection for nucleotide changes in enhancers (Whalen and Pollard, 2022), often involving exaptation of transposable elements (Barth et al., 2020), have contributed, for example, to the uniquely human aspects of brain development (Reilly et al., 2015; Mangan et al., 2022), thermoregulation (sweat glands in the skin) (Aldea et al., 2021), and digit and limb patterning, including the increase in size and rotation of the thumb towards the palm for enhanced dexterity (Prabhakar et al., 2008). Many loci contain clusters of enhancers with overlapping activities that are deployed to produce specific patterns of gene expression in different cell types (Whiting et al., 1991; Li et al., 2002; Perry et al., 2011; Malkmus et al., 2021), and it is clear that body plan specification is controlled by multiple enhancers to ensure precise patterns of gene expression during development (Woltering and Duboule, 2010; Perry et al., 2011), the robustness of which is increased by additional so-called "shadow" enhancers that confer increased precision and may provide buffering against noise (Hong et al., 2008; Cannavò et al., 2016; Osterwalder et al., 2018; Waymack et al., 2020; Kvon et al., 2021).

The mechanism of enhancer action.

The mechanism of enhancer action has long been a matter of speculation. A popular model is that put forward in the mid-1980s to reconcile enhancer function with transcription factor control of gene expression, which posits that transcription factor binding sites located in the enhancer are brought into contact with target protein-coding gene promoters by long-distance DNA looping (Dynan and Tjian, 1985; Ptashne, 1986; 1988; Popay and Dixon, 2022). This "crosstalk" model has been described as an example of a founder fallacy, whereby an initial interpretation of limited data is accepted and becomes subject to validation creep (Halfon, 2019).

It is well established that enhancer action alters chromatin topology and the juxtaposition of distal chromosomal sequences in 3-dimensional space, with consequent transcriptional activation of genes in their orbit (Larke et al., 2021; Popay and Dixon, 2022). Enhancer-mediated DNA "loops" may be equivalent to topologically-associated domains (TADs) identified by chromatin conformation capture analysis (Rao et al., 2014; Symmons et al., 2014; Lupianez et al., 2015; Hansen et al., 2018; Souaid et al., 2018) and play a role in the formation and organization of such domains (Furlong and Levine, 2018; Souaid et al., 2018; Lim and Levine, 2021). TAD boundaries are maintained by the interplay of the Cohesin and Mediator complexes and the zinc finger "transcription factor" CTCF (Kagey et al., 2010; Lee and Iyer, 2012; Wutz et al., 2017; Hansen et al., 2018; Popay and Dixon, 2022), all of which bind RNA or RNA/DNA hybrids (Yao et al., 2010; Saldana-Meyer et al., 2014; Kung et al., 2015; Hansen et al., 2019; Pan et al., 2020; Kuru-Schors et al., 2021). Enhancers also play a role in the etiology of complex diseases, developmental disorders and cancer (Hnisz et al., 2013; Smith and Shilatifard, 2014; Herz, 2016; Chen and Liang, 2020; Jindal and Farley, 2021), and disruptions of chromatin topological domains lead to rewiring of gene-enhancer interactions with pathogenic consequences (Smith and Shilatifard, 2014; Lupianez et al., 2015; Krijger and de Laat, 2016; Souaid et al., 2018; Nott et al., 2019; Beagan et al., 2020; Nasser et al., 2021).

There is now considerable evidence that enhancer-driven loop/TAD formation involves the formation of phase-separated biomolecular condensates that act as transcription/splicing hubs (Hnisz et al., 2017; Boehning et al., 2018; Boija et al., 2018; Cho et al., 2018; Hahn, 2018; Sabari et al., 2018; Fang et al., 2019; Nair et al., 2019; Shrinivas et al., 2019; Wang et al., 2019; Jia et al., 2021; Lim and Levine, 2021; Wang J et al., 2021), which are disrupted in developmental disorders and cancer (Kaiser and Semple, 2017; Ahn et al., 2021). *In vivo,* phase-separated domains (PSDs) are formed by interactions between RNAs and proteins containing intrinsically disordered domains (IDRs) (Järvelin et al., 2016; Fay and Anderson, 2018; Polymenidou, 2018; Protter et al., 2018; Garcia-Jove Navarro et al., 2019; Sanders et al., 2020; Roden and Gladfelter, 2021), which include almost all transcription factors, chromatin-modifying proteins, splicing factors, other RNA binding proteins, Mediator and other complexes involved in TAD formation (Gueroussov et al., 2017; Boija et al., 2018; Hentze et al., 2018; Niklas et al., 2018; Watson and Stott, 2019; Richter et al., 2022), the incidence of which correlates with developmental complexity (Yruela et al., 2017; Kulkarni and Uversky, 2018; Niklas et al., 2018). IDRs determine the localization of transcription factors to

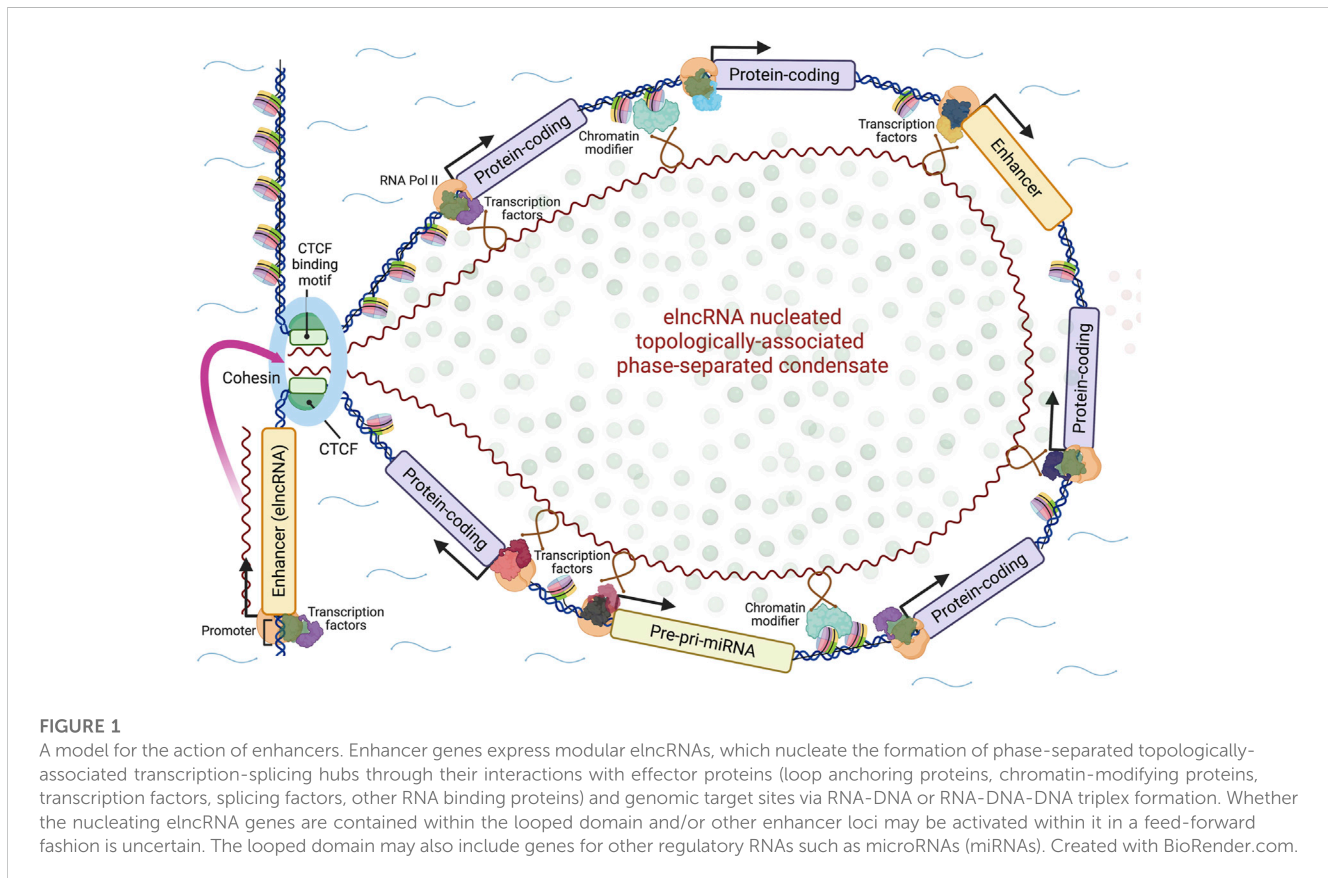

FIGURE 1
A model for the action of enhancers. Enhancer genes express modular elncRNAs, which nucleate the formation of phase-separated topologically-associated transcription-splicing hubs through their interactions with effector proteins (loop anchoring proteins, chromatin-modifying proteins, transcription factors, splicing factors, other RNA binding proteins) and genomic target sites via RNA-DNA or RNA-DNA-DNA triplex formation. Whether the nucleating elncRNA genes are contained within the looped domain and/or other enhancer loci may be activated within it in a feed-forward fashion is uncertain. The looped domain may also include genes for other regulatory RNAs such as microRNAs (miRNAs). Created with BioRender.com.

target promoters *in vivo* (Brodsky et al., 2020), and are overrepresented in alternatively spliced exons subject to tissue- and lineage-specific regulation (Romero et al., 2006; Buljan et al., 2012). IDRs are also the major sites of post-translational modifications (Bah and Forman-Kay, 2016), which influence their interactome or "promiscuity" (Cumberworth et al., 2013; Niklas et al., 2015; Wright and Dyson, 2015; Protter et al., 2018; Balcerak et al., 2019; Macossay-Castillo et al., 2019), a central feature of their flexibilities and capabilities, essential to cell state specification during development (see below). Nearly half of RNA-binding sites map to IDRs, which are hotspots of disease due to missense, nonsense and frame-shift mutations (Vacic and Iakoucheva, 2012; Hentze et al., 2018; Meyer et al., 2018; Tsang et al., 2020; Ahmed et al., 2022).

However, there is no evidence that transcription factors bound to enhancer sequences contact the promoters of target genes; rather, this is an enduring assumption. By contrast there is extensive evidence that enhancers are transcribed in the cells in which they are active (Hnisz et al., 2013; Arner et al., 2015; Kim et al., 2015; Li et al., 2016; Hon et al., 2017; Arnold et al., 2020).

Transcription from enhancers

A large fraction of "extragenic" RNA pol II transcription sites overlap enhancers (De Santa et al., 2010). Enhancers express short unstable bi-directional RNAs (sometimes called "eRNAs") (Koch et al., 2011; Azofeifa et al., 2018). However, short bi-directional unstable RNAs are also produced from active protein-coding genes, and likely have a function in both cases (Seila et al., 2008; Young et al., 2017). Indeed, the epigenetic landscape of, and the features of transcription initiation at, the promoters of enhancers and protein-coding genes are almost indistinguishable (Koch et al., 2011; Core et al., 2014; Arner et al., 2015; Kim et al., 2015; Li et al., 2016; Arnold et al., 2020). Both protein-coding genes and enhancers express long multi-exonic RNAs (Wu et al., 2014; Arner et al., 2015; Kim et al., 2015; Gil and Ulitsky, 2018; Carullo et al., 2020; Sartorelli and Lauberth, 2020), and may not be mutually exclusive, noting the complexity and interleaved nature of mammalian transcription and the fuzzy boundaries of genes (Mattick, 2003; Engstrom et al., 2006; Kapranov et al., 2007), the observations that many enhancers are located in introns or antisense to protein-coding genes (Gillies et al., 1983; Hong et al., 2008; Borsari et al., 2021; Thomas et al., 2021; Bachu et al., 2022), that intronic RNAs constitute the major fraction of the non-coding non-ribosomal RNA in mammalian cells (St Laurent et al., 2012), that antisense RNAs regulate development-specific alternative splicing (Degani et al., 2021; Pérez-Lluch et al., 2021), and that the major transcript from an estimated 17% of human protein-coding genes lacks an annotated coding sequence as indicated by GENCODE (Gonzàlez-Porta et al., 2013).

The long transcripts expressed from enhancers have also been referred to as "eRNAs," a term that should be reserved for them given that the short bidirectional transcripts also called eRNAs are not specific to enhancers but simply indicative of active promoters. However, to avoid confusion and provide specificity, a better descriptor is "elncRNAs" (enhancer-derived long noncoding RNAs) (Setten et al., 2021; Tan and Marques, 2022), which will

be used henceforth. It has been shown that elncRNAs are transcribed by RNA Polymerase II and retained in the nucleus (Koch et al., 2011; Natoli and Andrau, 2012), and that RNA exosome-regulated long non-coding RNA transcription controls super-enhancer activity (Pefanis et al., 2015).

Enhancer transcription is considered the best molecular indicator of enhancer activity in developmental processes (Wu et al., 2014; Arner et al., 2015; Kim et al., 2015; Carullo et al., 2020; Sartorelli and Lauberth, 2020) and cancers (Chen and Liang, 2020). There has been uncertainty about whether the resulting eRNAs are byproducts of TF binding at enhancers or are integral to enhancer action (Li et al., 2016; Schoenfelder and Fraser, 2019), but the evidence is now strongly in favor of the latter, although it is also clear that the act of transcription itself modulates enhancer activity (Pande et al., 2018; Morf et al., 2020; Pande et al., 2020). While genetic analysis by enhancer deletion may not distinguish between the loss of cis-acting DNA regulatory elements and trans-acting elncRNAs (Gao et al., 2020; Andergassen and Rinn, 2021), more incisive strategies such as the insertion of polyA transcription termination sites, removal of elncRNA exons, siRNA- and CRISPRi/Cas13-directed RNA knockdown have shown that elncRNAs are required for enhancer function in diverse contexts (Maass et al., 2012; Li et al., 2013; Melo et al., 2013; Lam et al., 2014; Paralkar et al., 2014; Sun et al., 2014; Xiang et al., 2014; Yin et al., 2015; Yang et al., 2016; Isoda et al., 2017; Cajigas et al., 2018; Groff et al., 2018; Fatima et al., 2019; Lewandowski et al., 2019; Allou et al., 2021; Andergassen and Rinn, 2021; Cajigas et al., 2021; Setten et al., 2021; Zibitt et al., 2021). Structural probing and mutational analysis has shown that the elncRNA *MUNC*, like lncRNAs generally (Mattick et al., 2023), has a defined secondary structure with separate domains that mediate different functions during myogenesis (Przanowska et al., 2022). Indeed, there is strong evidence that lncRNAs have a modular structure, often involving "repeat sequences," capable of scaffolding chromatin-modifying and other proteins and interacting with genomic target sites via R-loop or triplex formation (Brosius, 2014; Mattick et al., 2023).

Ectopic expression of elncRNAs increases expression of genes targeted by the enhancer (Alvarez-Dominguez et al., 2017; Shii et al., 2017), although there are exceptions (Anderson et al., 2016), and both splicing and modification of elncRNAs modulate enhancer activity (Li et al., 2015; Gil and Ulitsky, 2018; Tan et al., 2020; Lee et al., 2021; Tan and Marques, 2022). Reciprocally, a number of long noncoding RNAs (lncRNAs) have been shown to emanate from enhancers (Koch et al., 2011; Kim et al., 2015) and/or have enhancer-like developmental effects (Orom et al., 2010; Luo et al., 2016; Alexanian et al., 2017; Barter et al., 2017; Deveson et al., 2017; Liu et al., 2017; Micheletti et al., 2017; Andersen et al., 2019; Field et al., 2019; Ritter et al., 2019; Wilson et al., 2020; Dill et al., 2021; Pal et al., 2021; Wu et al., 2022). Genome-wide enhancer maps have linked disease risk variants to loci identified by genome-wide association studies (Nasser et al., 2021), most of which express lncRNAs (Bartonicek et al., 2017; Hardwick et al., 2019).

There is a striking degree of congruence between the features of elncRNAs and lncRNAs generally (Natoli and Andrau, 2012; Wu et al., 2014; Mattick et al., 2023). Transcriptional data suggest that many if not most lncRNAs are derived from enhancers (Li et al., 2016; Hon et al., 2017). Their numbers are broadly similar: although there are only –20,000 human lncRNA genes annotated in GENCODE[1], –100,000 human lncRNA genes have been catalogued in dedicated databases (Fang et al., 2017; The RNAcentral Consortium, 2018; Ma et al., 2019; Volders et al., 2019; Statello et al., 2021); there are likely many more (Deveson et al., 2017), given the under sampling of cells at different developmental stages and the high resolution analyses that have revealed the existence of previously unreported lncRNAs and their isoforms expressed from GWAS regions (Bartonicek et al., 2017; Hardwick et al., 2019), across ch21 (Deveson et al., 2018) and from well-characterized loci, such as those containing *p53* and *HOX* genes (Mercer et al., 2012). While some may be a product of transcriptional noise (Brosius and Raabe, 2016; Xu et al., 2023), thousands of lncRNAs (including many "antisense" RNAs) have been shown to have biological effects when their sequence or expression is perturbed and, while most have not been investigated, indices of their functionality include differential expression, subcellular localization, promoter and splice site conservation, multiexonic structure and extensive alternative splicing (Mattick and Amaral, 2022; Mattick et al., 2023), the latter shown to affect enhancer activity (Tan and Marques, 2022). Like lncRNAs generally (although there are exceptions, such as lncRNAs associated with more generic subnuclear domains such as nucleoli, paraspeckles and neuronal granules (Yamazaki et al., 2021; Yamazaki and Hirose, 2021; Grzejda et al., 2022)), elncRNAs are expressed at relatively low levels and exhibit only modest conservation across species (Deveson et al., 2017; Sartorelli and Lauberth, 2020; Mattick et al., 2023), features consistent with cell- and lineage-specific regulatory functions.

Chromatin modification

High resolution imaging shows the localization of many lncRNAs in punctate domains in the nucleus (Cabili et al., 2015; Quinodoz et al., 2021), with other studies showing widespread chromatin targeting of lncRNAs (Mishra and Kanduri, 2019) and the involvement of lncRNAs [sometimes referred to as "architectural" RNAs (Takeshi et al., 2017)] in the organization of chromosome territories via phase separation (Redrup et al., 2009; Cerase et al., 2019; Li and Fu, 2019; Pessina et al., 2019; Thakur and Henikoff, 2020; Wu et al., 2021a; Bridges et al., 2021; Elguindy and Mendell, 2021; Luo et al., 2021; Wang R et al., 2021; Zhu et al., 2021). Cohesin, Mediator and CTCF, which determine TAD boundaries, have been shown to be recruited to their target sites by enhancer-derived RNAs (Lai et al., 2013; Li et al., 2013; Tsai et al., 2018; Schoenfelder and Fraser, 2019; Islam et al., 2023). Enhancer function has been shown to require assembly of an enhancer RNA–dependent ribonucleoprotein condensate (Nair et al., 2019), and Mediator complexes with cohesin to form rings that connect two DNA segments and clusters with RNAPII in transcription-dependent condensates (Kagey et al., 2010; Cho et al., 2018). Enhancers also interact

1 https://www.gencodegenes.org/human/stats.html.

with histone modifying proteins and elncRNAs have been shown to modulate DNA and histone modifications and transcription factor binding (Bose et al., 2017; Carullo et al., 2020; Harrison and Bose, 2022). Similarly, lncRNAs associate with chromatin modifying complexes (Dinger et al., 2008; Nagano et al., 2008; Pandey et al., 2008; Khalil et al., 2009), including those identified as elncRNAs involved in the maintenance of stem cell fates and lineage specification (Dinger et al., 2008; Wang K C et al., 2011; Yang et al., 2014; Deng et al., 2016; Subhash et al., 2018).

RNA binding by chromatin-modifying complexes has—like that of IDR-containing proteins generally—been described as "promiscuous" (Davidovich et al., 2013), reflecting their ability to interact with many partners, determined by local concentration, alternative splicing, and post-transcriptional and post-translational modifications (Romero et al., 2006; Buljan et al., 2012; Weatheritt et al., 2012; Cumberworth et al., 2013; Deveson et al., 2018; Protter et al., 2018; Balcerak et al., 2019; Macossay-Castillo et al., 2019; Wu et al., 2021b; Shiau et al., 2022). Cytosine (5mC) and adenosine (m6A) modifications of elncRNAs are known features of active enhancers that regulate their abundance, facilitate transcriptional condensate formation and potentiate co-activator function (Aguilo et al., 2016; Lee et al., 2021; Xu et al., 2022). RNA modifying enzymes accompany elncRNAs in the formation of PSDs (Harrison and Bose, 2022) and RNA modifications affect a wide range of developmental processes (Mattick and Amaral, 2022). Interestingly, biomolecular condensates also play a role in RNA-directed transgenerational epigenetic inheritance (Wan et al., 2018).

A new model of enhancer action.

As noted already, the popular model for enhancer action posits that transcription factor binding sites located in the enhancer are brought into contact with target protein-coding gene promoters by long-distance DNA looping (Dynan and Tjian, 1985; Ptashne, 1986; Ptashne, 1988; Popay and Dixon, 2022), which is simple to conceive and illustrate. A new schema must consider all of the above information, including the formation of phase-separated topologically-associated domains and the guidance by elncRNAs of TAD boundary proteins, chromatin-modifying complexes and transcription factors to target sites within these domains by RNA-protein and RNA-DNA interactions. A model that attempts to do this is presented in Figure 1, with the important caveat that, while such models can illustrate and expose hypotheses to be tested, they can also constrain and mislead.

Conclusion

There are two unresolved major features of molecular biology and genetics: lncRNAs and enhancers, both estimated to number in the hundreds of thousands in mammals. The emergent picture is that 1) enhancers comprise their promoters and the RNAs that are transcribed from them and 2) a major subclass of lncRNAs is elncRNAs, whose function is to regulate chromatin architecture and thereby expression of protein-coding and other RNAs (including potentially miRNAs, antisense RNAs and other elncRNAs in a developmental feed-forward fashion), through physical mechanisms that involve recognition of transcription factors, chromatin-modifying complexes, RNA binding and other effector proteins containing IDRs, as well as their genomic target sequences via R-loops or triplexes (Soibam, 2017; Cetin et al., 2019; Mishra and Kanduri, 2019; Cai et al., 2020; Farabella et al., 2021), to form topologically-associated domains that act as developmental stage-specific transcriptional and splicing hubs.

This is not to say that all lncRNAs are elncRNAs; there are many others involved in the formation of specialized domains in the nucleus and the cytoplasm (including metabolic pathways, translation, synapse architecture and autophagy) (Lyon et al., 2021), most of which have yet to be characterized. It is also important to reiterate that, while enhancers and those encoding mRNAs are "genes" in the sense of producing products with phenotypic consequences, they are not discrete entities but rather entangled components of a continuum of genetic information. Moreover, protein-coding loci may also have enhancer activity (Engreitz et al., 2016) and, while it has been observed that transcription affects chromatin architecture (Mele and Rinn, 2016; Creamer et al., 2021), transcription alone does not provide the specificity needed for fine scale control of the epigenetic status and expression of nearby genes.

Enhancers must act transiently at almost every stage of ontogeny [see, e.g. (Bachu et al., 2022; Landshammer et al., 2023)], most of which have not been polled. Humans contain an estimated 30 trillion cells (excluding the microbiome) (Bianconi et al., 2013; Sender et al., 2016), which means that -6×10^{13} binary cell fate (differentiate and/or divide) decisions must be made with high precision and reproducibility to ensure the correct formation of an adult with its myriad of architecturally distinct and correctly wired muscles, bones and organs, exemplified by the phenotypic congruence of monozygotic twins (Mattick and Amaral, 2022).

This number of cell fate decisions is four orders of magnitude greater than the linear information content of the human genome (about $\times 610^9$ bits), which implies a dense file structure (Mattick and Amaral, 2022) that may be unzipped by combinatorics of the extensive alternative splicing of lncRNAs (Mercer and Mattick, 2013; Deveson et al., 2018; Rinn and Chang, 2020), RNA modifications, and post-translational modifications of IDRs in interacting proteins and their alternatively spliced exons (Romero et al., 2006; Buljan et al., 2012) to guide the formation, composition and genomic sphere of influence of enhancers at each stage of developmental ontogeny.

It will be a mammoth challenge to decipher the feed-forward program that determines enhancer expression, the structure-function relationships and interactome of elncRNAs during development. It will be an even bigger challenge to understand how regional enhancer action is integrated to inform cell fate decisions, likely by the centrosome[2] in animals (itself a phase-separated organelle)

2 3′UTRs have been implicated in centrosome localization (Bergalet et al., 2020), an interesting observation in view of the fact that many if not most mammalian genes express 3′UTRs (separately from their normally associated protein-coding sequences) that are chromatin-localized, inhibit cell division and induce differentiation (Mercer et al., 2011; Kocabas et al., 2015; Vilborg et al., 2015), in one case essential for oocyte development (Jenny et al., 2006).

(Zwicker et al., 2014; Conduit et al., 2015; Joukov and De Nicolo, 2019) and the mitotic spindle in plants (Liu and Lee, 2022), noting that plant development is more flexible to be responsive to environmental circumstances. Whatever the details, it is evident that the fraction of the genome devoted to enhancers scales with developmental complexity, and that sequences specifying elncRNAs occupy a far greater fraction of animal and plant genomes than those specifying proteins.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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